Anal. Calcd. for $C_6H_{13}O_9P(C_{23}H_{26}N_2O_4)_2 \cdot 8H_2O$: H_2O, 12.1; C, 52.33; H, 6.85; N, 4.70; P, 2.60. Found: H_2O, 12.2; C, 52.7; H, 6.88; N, 4.57; P, 2.66.

The anhydrous form was likewise crystalline and was obtained by heating the octahydrate at 110° under reduced pressure over phosphorus pentoxide; m. p. 182–184° (dec.). The same dibrucine salt was obtained on starting with a dipotassium salt from either the synthetic or natural[1] source.

The crystalline dipotassium salt was regenerated from the above dibrucine salt. To 0.5 g. of the dibrucine salt dissolved in 5 cc. of water was added 0.8 cc. of 10% potassium hydroxide to bring the *p*H to about 8.4 (thymol blue). The brucine was removed by filtration and ethanol was added to the filtrate to incipient turbidity. The dipotassium salt crystallized on scratching and cooling; yield 0.14 g. (93%), spec. rot. +78° (33°, *c* 1, 5892.5 Å., H_2O). This rotation is in exact agreement with the previously published value[3] (+78°).

Hydrolysis of the α- and β-Forms of *d*-Glucopyranose Dibrucine 1-Phosphate.—The two previously described dibrucine salts of *d*-glucopyranose 1-phosphate were hydrolyzed at room temperature with *N* hydrochloric acid, and the course of the reactions followed polarimetrically (Fig. 3). The rotation change (anhydrous basis) of the α-form was +9° (extrapolated) → −14° while that of the β-form was −21.5° (extrapolated) → −14°. The specific reaction constant at 33° of the α-form was *ca.* 0.005 while that of the β-form was *ca.* 0.015 (minutes and decimal logarithms) when calculated according to the equation $k = 1/t \log (r_0 - r_\infty / r_t - r_\infty)$ wherein t is time, r_0 is initial rotation, r_∞ is final rotation and r_t is rotation at time t. Thus the β-form is appreciably more sensitive to acidity than is the α-form. Using the value of +53° for the specific rotation of *d*-glucose in *N* hydrochloric acid[7] and a determined value of −26.4° for the specific rotation of levorotatory brucine under the same conditions, the calculated specific rotation for the final hydrolyzate may be obtained from the equation: [M]/1048 = (+53)(180) + 2(−26.4)(466) = −14.4°, wherein [M] is molecular rotation. The value of the specific rotation thus calculated is in agreement with the determined value of −14.2° (anhydrous basis).

We are indebted to Mr. Walter M. Anderson for assistance in the laboratory.

Summary

1. It is shown that the Cori ester is the α-form of *d*-glucopyranose 1-phosphate, by the synthesis of its α,β-isomer, characterized as its crystalline dibrucine salt.

2. The crystalline dibrucine salt of the Cori ester has been characterized.

3. It is shown that both of the above dibrucine salts are stable to alkali but sensitive to acid, with the β-form being even more acid-labile than the α-form.

(7) M. L. Wolfrom and L. W. Georges, THIS JOURNAL, **59**, 282 (1937).

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[CONTRIBUTION FROM THE U. S. BUREAU OF NARCOTICS LABORATORY]

Isolation of a Physiologically Active Tetrahydrocannabinol from Cannabis Sativa Resin

BY H. J. WOLLNER, JOHN R. MATCHETT,[1] JOSEPH LEVINE AND S. LOEWE[2]

Greatly increased interest has developed in recent years in the chemistry and pharmacology of the oil derived from *Cannabis sativa*. The researches initiated have greatly extended the previous knowledge of the complex mixture, which has been ably reviewed by Blatt.[3]

Adams and co-workers, in a series of brilliant researches, have rigidly proven the structure of cannabinol,[4] isolated cannabidiol,[5] isomerized it to two isomeric, physiologically active tetrahydrocannabinols,[6] and proven its structure except for final placement of one double linkage.[7] The same workers have prepared synthetic tetrahydrocannabinols as well as a number of similarly constituted substances,[8] which have been shown to possess "marihuana-like" physiological activity in dogs, but in smaller degree than those prepared from the naturally-occurring cannabidiol.

Todd and his co-workers have isolated cannabol,[9] and in synthetic studies have recorded results similar to those reported by Adams.[10] The Geyer test was used to measure physiological activity of their synthetic material.

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(3) Blatt, *J. Wash. Acad. Sci.*, **28**, 465 (1938).

(4) Adams, Baker and Wearn, THIS JOURNAL, **62**, 2204 (1940).

(5) Adams, Hunt and Clark, *ibid.*, **62**, 196 (1940).

(6) (a) Adams, Pease, Cain and Clark, *ibid.*, **62**, 2402 (1940). (b) Adams, Cain, McPhee and Wearn, *ibid.*, **63**, 2209 (1941).

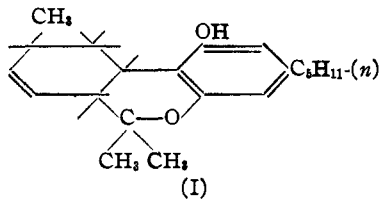
(7) Most recent paper of series, Adams, Loewe, Pease, Cain, Wearn, Baker and Wolff, *ibid.*, **62**, 2566 (1940).

(8) Most recent paper of series, Adams, Cain and Loewe, *ibid.*, **63**, 1977 (1941).

(9) Jacob and Todd, *Nature*, **145**, 350 (1940).

(10) (a) Ghosh, Todd and Wilkinson, *J. Chem. Soc.*, 1121 (1940). (b) Ghosh, Todd and Wright, *ibid.*, **137** (1941). (c) Russell, Todd, Wilkinson, Macdonald and Woolfe, *ibid.*, 169 (1941).

The natural tetrahydrocannabinol (I) molecule as formulated by Adams contains three asymmetric



(I)

carbon atoms, and the position of the alicyclic double bond is not fixed. It is thus apparent that many isomers are possible, any or all of which may occur naturally. The relative potency of the wholly synthetic products and those prepared from cannabidiol indicates that these may vary a great deal in physiological activity. It is not unreasonable to suppose that "red oil" is composed essentially of cannabinol, cannabidiol and various isomeric tetrahydrocannabinols, the proportions of which may vary widely. One might thus account for the extremely variable character of cannabis extracts and of "red oil" which has long been observed pharmacologically, and which is frequently referred to in the chemical literature. In this Laboratory we have observed wide variation in the character of "red oil" prepared from different source materials, and have found that variation in response to the alkaline Beam test (cannabidiol content) is related to difference in agronomic variety of cannabis.[11] It is of concurrent interest to note the remarkable constancy in analytical data and reactions of "crude cannabinol" or "red oil" which led early workers to the opinion that the substance was a chemical individual.[3]

It is the purpose of this paper to describe the separation, from oil derived from Indian charas, of a tetrahydrocannabinol evidently homogeneous, and which is very potent physiologically as manifested by response in dogs. The substance, isolated as the acetate, is a colorless, viscous, optically active oil. Two reactions, in addition to analytical data, provide the key to the structure, though they do not, of course, furnish evidence establishing the position of the double bond. It rapidly absorbs one mole of hydrogen in acetic acid solution in the presence of Adams platinum catalyst, and is readily dehydrogenated by chloranil in boiling xylene, toluene, or chlorobenzene, or by sulfur at 225°, to cannabinol acetate.

In the absence of crystalline derivatives we have, perforce, based our judgment of homogeneity on inability to further partition the material by means which have proven efficient in separating it from mixtures with closely related substances.

The separation, following acetylation of the crude oil, was essentially accomplished by selective adsorption, first by passage over silica gel in benzene solution to remove cannabidiol diacetate and substances of unknown character, then over activated alumina in carbon tetrachloride solution and in pentane solution to remove substances of lower optical rotation. The rotation was not increased by a further passage over an alumina column.

The product was distilled in a specially designed high-vacuum fractionating column into four main fractions (*ca.* 85%) and small heads and tails fractions. The four principal fractions proved identical in physical and pharmacological properties. A composite prepared from them was passed over a fresh activated alumina column in pentane solution and the unadsorbed portion recovered with unchanged properties.

Under these latter conditions a considerable portion of the material was adsorbed. Upon elution with alcohol, it was found to be markedly lower in optical rotation and in physiological potency than the unadsorbed portion. The character of this change has not been studied. On standing, especially in thin layers in contact with air, the material develops a yellow color and its rotation and potency drop. Analyses indicate that these changes are due to oxidation.

The acetate is readily hydrolyzed by acid, alkali, or ammonia in alcoholic solution. The deacetylated product has in each case a different specific rotation and a lower physiological potency than the acetate. Reacetylation does not restore either of these properties to its former value.

TABLE I

Substance	$n^{20}D$	$[\alpha]^{27}D$	Absorption spectra: Peak, Å.	Log ϵ	Peak, Å.	Log ϵ
Low-rotating tetrahydrocannabinol (Adams)[6b]	1.5425	−130°	2745	3.17	2815	3.21
High-rotating tetrahydrocannabinol (Adams)	1.5440	−265°	2745	3.17	2815	3.21
Present product	1.5500	−193°	2760	3.42	2820	3.43

Bioassays were made by the "Method of Approximation" already reported.[12]

(11) Matchett, Levine, Benjamin, Robinson and Pope, *J. Am. Pharm. Assoc.*, **29**, 399 (1940).

(12) (a) Loewe, *ibid.*, **28**, 427 (1939); (b) Loewe, *J. Pharm. Exptl. Therap.*, **66**, 23 (1939); (c) Matchett and Loewe, *J. Am. Pharm. Assoc.*, **30**, 130 (1941).

A sample of the acetate furnished to Roger Adams and C. K. Cain was kindly ammonolyzed by them in toluene. The resulting tetrahydrocannabinol analyzed correctly, and differed in rotation and refractive index from either of those prepared by Adams through isomerization of cannabidiol. Ultraviolet absorption was similar in all wave lengths, but of greater intensity. Table I records the various properties.

Potency values are reported in Table II as compared with 1-hydroxy-3-*n*-amyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-benzopyran[13] as standard.[14]

Experimental

Preparation of Crude Distillate.—The charas used in this investigation was of Indian origin, received in the Fall of 1939. Alcohol extraction in the cold yielded a viscous brown oil amounting to about 30% of the weight of the starting material. The residue was a grayish sandy-appearing powder containing many plant hairs. The oil after removal of alcohol *in vacuo* was taken up in Skelly Solve D, and the solution filtered. This solvent was removed, and the oil distilled *in vacuo* in a cascade-type still which will be described separately. The "crude distillate" so obtained was a reddish-brown very viscous oil; rotation, $[\alpha]^{21}$ −120° in alcohol.[15]

Acetylation of Crude Distillate.—The crude distillate was readily acetylated by the method of Cahn[16] or by boiling for two hours with approximately three volumes of acetic anhydride.[17] The resulting acetate is more fluid than the original material and increased in weight by about 25%. No difference was noted in the products resulting from the two procedures. Each was carefully fractionated (still temperature 140–210°, (0.001 mm.) McLeod gage) in a molecular still. Cannabinol acetate did not crystallize from any of the fractions after solution in alcohol and long standing in the refrigerator.

Partition of the Acetylated Mixture by Selective Adsorption.—(A) The material used was acetylated with acetic anhydride and fractionally distilled *in vacuo* to remove heads and tails; about half was so distilled prior to, and the remainder subsequent to, acetylation. A solution of 320 g. of this in benzene (2 g. per 100 ml.) was passed over silica gel (150–200 mesh) in 35-mm. columns, 500 mm. in length. After 25 g. (1250 ml.) had been passed over each column, it was followed by pure benzene until the effluent contained a trace of cannabidiol diacetate as evidenced by a faint positive response to the alkaline Beam test. This process required 700–800 ml. of benzene. The washings and original effluent solutions were combined and the solvent removed *in vacuo*. The recovery amounted to 187 g. of a light yellow oil somewhat more fluid than the original mixture; rotation, $[\alpha]^{21}$ −180°.

The material remaining on the columns was recovered by elution with alcohol; it contained much cannabidiol (alkaline Beam test).

(B) The columns described above were charged with a mixture of 100 g. of Alorco Grade A 150–200-mesh activated alumina and 50 g. of Hi-Flo Super-Cel. The unadsorbed acetate (180 g.) obtained as described under (A) was dissolved in carbon tetrachloride (2 g. per 100 ml.), and 30 g. passed over each column. The columns were washed with carbon tetrachloride to virtual exhaustion (700 ml. each). With the exception of a light yellow-brown band (50 mm.) at the top of each column, no significant bands were in evidence either in daylight or under an ultraviolet lamp. Recovery amounted to 135 g.; rotation, $[\alpha]^{21}$ −195°. The adsorbed material was eluted by alcohol and set aside.

(C) Columns were prepared in the fashion described under (B) and the product passed through them dissolved in Skelly Solve A (2 g. per 100 ml.), until 30 grams had been passed through each. The columns were washed to practical exhaustion with Skelly Solve A (1000 ml. each). The solvent was removed *in vacuo*. Recovery amounted to 81 g.; rotation, $[\alpha]^{21}$ −205°. Small samples showed no significant rise in rotation when passed in the same way over similarly constituted small columns.

Fractional Distillation under High Vacuum.—The material described under (C) was separated into six fractions by distillation in a specially designed high-vacuum fractionating column which will be described elsewhere. The four principal fractions were a water-white viscous oil. The properties of all the fractions are collected in Table II.

TABLE II

Still charge, 75 grams; vacuum (McLeod gage) 0.015 mm.; temperature of charge during distillation of four principal fractions 141–142°.

Fraction	Grams	R. I. at 40°	Specific rotation	Potency
1	2.2	1.5018	− 93°	2.3 ± 10%
2	16.5	1.5193	−212°	13.5 ± 20%
3	14.0	1.5193	−213°	12.6 ± 20%
4	14.0	1.5194	−213°	13.1 ± 10%
5	15.7	1.5196	−211°	12.4 ± 23%
6	6.9	1.5204	−197°	3.1 ± 35%

Tetrahydrocannabinol Acetate.—A composite was prepared by mixing 5-g. portions from each of fractions 2, 3, 4 and 5 and used at once for the indicated experiments described below.

Anal. Calcd. for $C_{23}H_{32}O_3$: CH_3CO, 12.08. Found: CH_3CO, 12.07.[18]

A solution of 1.5 g. of the freshly prepared composite in 150 ml. of pentane was passed over a column charged with 35 g. of alumina–Super-Cel mixture referred to above. A total of 700 ml. of pentane was used to wash the unadsorbed material through. Recovery amounted to 0.43 g.;

(13) Adams and Baker, THIS JOURNAL, **62**, 2405 (1940).

(14) This synthetic tetrahydrocannabinol is given preference over the standard used in other papers (*cf.* ref. 7, 12c), an oil prepared by fractional distillation of "red oil" derived from cannabis grown in Minnesota. The activity of this oil is 4.35 referred to the new standard preparation. Use of this new standard is in accordance with more recent publications on the subject,[5] and the preference seems justified because the synthetic tetrahydrocannabinol is a uniformly reproducible substance.

(15) Optical rotations, except where otherwise noted, were determined using a bichromate filter, according to "Methods of Analysis of the Assoc. Official Agr. Chem.," 5th edition, 1940, p. 489.

(16) Cahn, *J. Chem. Soc.*, 630 (1931).

(17) Wood, Spivey and Esterfield, *ibid.*, **75**, 20 (1899).

(18) Method of Matchett and Levine, *Ind. Eng. Chem., Anal. Ed.*, **13**, 98 (1941).

$[\alpha]^{21}$ −214°; physiological potency, 14.6 ± 7.2%. The column was eluted with 300 ml. of carbon tetrachloride, yielding 0.19 g., and then with 300 ml. of acetone; 0.77 g. was recovered here; $[\alpha]^{21}$ −180°. This elutate had a potency of 2.0.

The material gradually developed a yellow color, especially when exposed in thin layers to the air. After standing for three months, careful redistillation of the composite yielded a principal fraction (90%) practically water-white, and a less volatile one which was bright yellow. Samples of each were submitted to Roger Adams and C. K. Cain, to whom we are indebted for the analytical and physical data.

Anal. Fraction 1 (tetrahydrocannabinol acetate). Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.46; H, 9.17. Rotation: $[\alpha]^{23}D$ −209°. $n^{20}D$ 1.5285. Absorption spectrum: peaks at 2745 (log e = 3.52) and 2805 (log e = 3.53). Fraction 2: Found: C, 75.18; H, 8.97. Rotation: $[\alpha]^{30}D$ −161°.

Dehydrogenation of Tetrahydrocannabinol Acetate.—(A) 0.9 gram of the freshly prepared composite referred to above was heated at 225° for thirty minutes with 0.35 g. of sulfur. The cooled mixture was extracted with alcohol. After removal of this solvent, the material was taken up in ethyl acetate and treated with Darco. Cannabinol acetate was recrystallized from alcohol, m. p. 75.5°;[15] yield, 0.55 g. (62%). Admixture with a sample of authentic cannabinol acetate, kindly furnished by Dr. Roger Adams, did not change the melting point.

(B) 1.1 grams of the material was refluxed for three hours with 1.7 g. of chloranil in 15 cc. of xylene. The xylene solution was washed first with 5% aqueous potassium hydroxide solution, then with water, and evaporated. The product was treated with Darco in alcohol and in ethyl acetate solution. The yield of cannabinol acetate was 0.7 g. (64%).

Hydrogenation of Tetrahydrocannabinol Acetate.[19]—A solution of 1.02 g. of the freshly prepared composite in 25 cc. of glacial acetic acid was treated with hydrogen at atmospheric pressure in the presence of 0.2 g. of Adams platinum catalyst. After five minutes, during which 76 cc. (1.05 moles) of hydrogen at 26° had been absorbed, the rate of absorption slowed to substantially zero.

(19) We wish to thank Dr. H. L. Haller for his assistance in carrying out the hydrogenation and for the use of his apparatus.

The solution was diluted with water and extracted with Skelly Solve A. Removal of the solvent left a water-white viscous oil; rotation, $[\alpha]^{21}$ −119°. The product has not as yet been further investigated.

Hydrolysis of Tetrahydrocannabinol Acetate

A. By Acid.—The residue from the determination of acetyl content of the acetate was recovered by diluting the acid alcoholic solution and extracting with petroleum ether; rotation, $[\alpha]^{21}$ −216°; potency, 8.04 ± 22%.

B. By Ammonia in Toluene.—A portion of the material referred to Adams and Cain was de-esterified according to the procedure described for cannabidiol bis-dinitrobenzoate[6]; the resulting tetrahydrocannabinol was separated by distillation into five fractions whose properties are indicated in Table III. The data which follow were kindly supplied by them.

Anal. Calcd. for $C_{21}H_{30}O_2$ (tetrahydrocannabinol): C, 80.21; H, 9.62. Found: C, 80.18; H, 9.87. $n^{20}D$ 1.5500 ± 0.0005. Absorption spectrum: peaks at 2760 (log e = 3.42) and 2820 (log e = 3.43).

TABLE III

Fraction	Wt.	$[\alpha]^{23}D$
1	0.23	−195°
2	.26	−193°
3	.93	−194°
4	.27	−185°
5	.80	−192°

The low rotation of fraction 4 has thus far not been accounted for.

The fractions were of a uniform golden yellow color which deepened somewhat on standing. The material was much more viscous than the acetate.

Summary

A tetrahydrocannabinol of high physiological potency, isomeric with but different from those prepared by isomerization of cannabidiol, has been isolated from "red oil" derived from charas of Indian origin.

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